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HORMESIS: IMPLICATIONS FOR CANCER RISK ASSESSMENT

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□   Current guidelines for cancer risk assessment emphasize a toxicant's "mode of action", rather than its empirically derived dose-response relationship, for determining whether linear low-dose extrapolation is appropriate. Thus, for reasons of policy, demonstration of hormesis is generally insufficient to justify a non-linear approach, although it may provide important insights into the actions of toxicants. We evaluated dose-response characteristics of four carcinogens reported to have hormetic dose-response curves: cadmium chloride; ionizing radiation; PAHs; and, 2,3,7,8-TCDD. For each, the study that documented hormesis in one organ also provided evidence of non-hormetic dose-responses in other organs or non-hormetic responses for seemingly similar carcinogens in the same species and organs. Such inconsistency suggests toxicologic reasons that the finding of hormesis alone is not sufficient to justify use of non-linear low-dose extrapolations. Moreover, available data in those examples are not sufficient to know whether hormesis is a property of the toxicants, the target organ, or the exposed species. From the perspectives of cancer risk assessment, the greatest informational value of hormesis may be that it provokes mechanistic studies intended to explain why hormesis occurs.

INTRODUCTION

Much has been written about the implications of hormesis for informing risk assessment (Calabrese *et al.*, 1999; Calabrese, 2004), but most attention has focused on its implications for the risk assessment of non-carcinogens, toxicants generally expected to have dose-response relationships characterized by low-dose thresholds. By contrast, the implications of hormesis for risk assessment of carcinogens have been subject to less detailed analysis. Of particular concern is whether the default risk assessment paradigm for cancer risk assessment, linearized low-dose extrapolation, is incompatible with the concept of hormesis.

That possibility raises interesting public health and science policy questions. The role of linearized low-dose extrapolation has been reaffirmed in the most recent US EPA Guidelines for Carcinogen Risk Assessment (US Environmental Protection Agency, 2003), which describe the toxicological conditions for which linearized extrapolation should be used:

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"Linear extrapolation should be used when there are data to indicate that the dose-response curve has a linear component below the POD, as when

- the agent is DNA-reactive and has direct mutagenic activity or the agent operates through another mode of action that is expected to be linear at low doses, or
- human exposure or body burden is high and near doses associated with key precursor events in the carcinogenic process, so that background exposures to this and other agents operating through a common mode of action are in the increasing, approximately linear, portion of the dose-response curve.

Linear extrapolation can also be used as a default approach when the available data fall short of establishing the mode of action at a tumor site, because linear extrapolation generally is considered to be a health-protective approach for addressing uncertainty about the mode of action".

By contrast, use of a "non-linear approach", presumably based on a threshold model, is restricted to those situations "when there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses and the agent does not demonstrate mutagenic or other activity consistent with linearity at low doses" (US Environmental Protection Agency, 2003).

To the extent that these Guidelines reflect science policy, it seems that there is little direct role for hormesis in carcinogen risk assessment. One reason for that is the central importance of a toxicant's "mode of action", rather than its empirically derived dose-response relationship, in determining an appropriate extrapolation model. The presence of hormesis might indicate a non-linear mode of action, but alternative explanations for hormesis include stimulation of cellular replication and upregulation of detoxication and repair mechanisms, processes likely to operate via modes of action that differ from carcinogenic mode of action.

In addition, for technical reasons of study design discussed below, standard NTP-type animal bioassays are unlikely to detect hormetic responses where they might otherwise exist. Nevertheless, hormetic responses have been described for a variety of carcinogens reflecting various modes of action. The question of how these observations inform risk assessment for such toxicants, however, remains unanswered.

DESIGN OF BIOASSAYS

Standard NTP-type animal bioassays cannot detect hormetic dose-responses if they are designed 'properly'. Such bioassays are usually conducted with four groups of about 50 animals per sex, including one unexposed control group, one high-dose group exposed at about the maximum tolerated dose (MTD), and two intermediate-dose groups exposed

to about 50% MTD and 25% MTD respectively. That design permits detection of high-dose effects, generally those that cause a statistically significant increased disease incidence greater than 5-10% over background. But hormesis is a low-dose phenomenon that cannot be observed without looking at low-dose exposures. Moreover, it's demonstration generally requires that studies include multiple low-dose exposures. For example, review of the Hormesis Database has indicated that to have a "reasonable chance" of demonstrating hormesis, "a study often requires at least six total doses, with at least three doses within a factor of 10 below the NOAEL" (Calabrese *et al.*, 1999). Standard bioassays do not have such a study design.

In consideration of the costs of standard bioassays as well as the customary risk-reduction focus of public health policy, it is understandable that most cancer bioassays have focused on high-, rather than low-dose exposures. Moreover, there is no obvious incentive for most agencies and public health planners to expand those bioassays in order to determine whether hormesis is a frequent or infrequent characteristic of the dose-response properties of carcinogens. Still, the demonstration of hormesis in a variety of cancer bioassays raises the possibility that if looked for, it might often be found. The challenge to toxicologists and public health authorities is to consider the processes likely to underlie such dose-response relationships and to determine those of direct relevance to risk assessment.

CADMIUM CHLORIDE

A carcinogen for which evidence has been cited of a hormesis-like response is cadmium chloride. Although the human cancer data remain in dispute, there is sufficient evidence of its carcinogenicity in animals. Cadmium has been associated with increased rates of testicular, prostate and lung cancer(National Toxicology Program, 2003; Waalkes *et al.*, 1992). Although its mode of action is not certain, direct DNA-reactivity seems unlikely. Most mutagenicity tests have been negative and studies in of mammalian cells generally show little or no genotoxicity (Hengstler *et al.*, 2003; Hartwig, 1995); *in vitro* studies, however, have demonstrated DNA cross-links, DNA strand breaks and frameshift mutations (Waalkes *et al.*, 1992; Sunderman, 1986; Waalkes and Poirier, 1984).

Figure 1 presents data for testicular and prostate tumors in Wistar rats exposed to cadmium chloride by a single sc injection at doses ranging from 0-40 μmol/kg (Waalkes *et al.*, 1988) and then observed for 104 weeks. Because there were six active doses plus a control and because of a positive background rate of tumors, the study design was optimal for demonstrating hormesis. It can be seen that testicular tumors demonstrate a hormetic response, but prostate tumors do not. If hormesis were a characteristic of cadmium, then it is not clear why different tissues would reflect such differing response profiles.
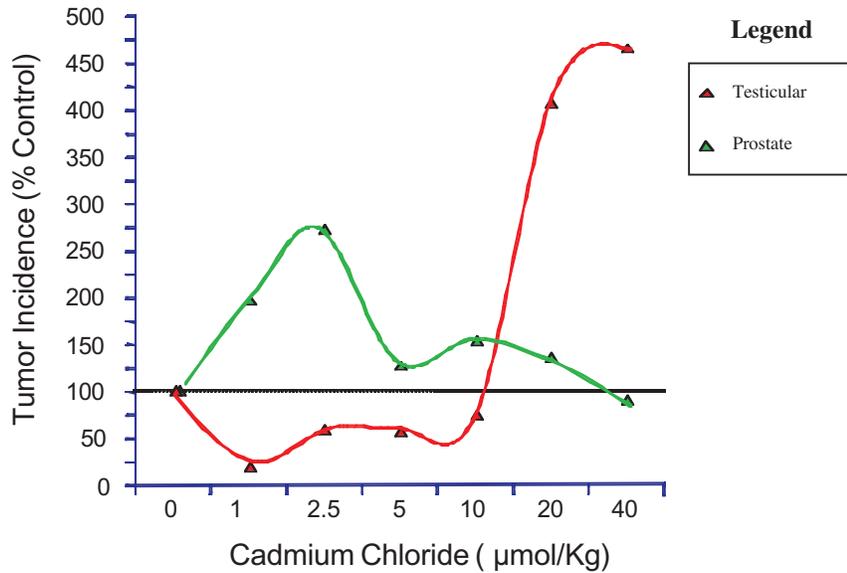
FIGURE 1 Incidence data for testicular and prostate tumors in Wistar rats exposed to cadmium chloride by single SC injection and followed until death {22332}.

IONIZING RADIATION

The hormetic effects of ionizing radiation have long been recognized (Luckey, 1980; Luckey, 1991), although not so long as its carcinogenic effects. Those early reports of hormesis did not specifically consider carcinogenicity, but contemporaneous experiments suggested a hormetic carcinogenic response in RFMf/Un mice exposed to graded doses of to (-irradiation (Ullrich and Storer, 1979). In that study, Ullrich and Storer exposed animals to radiation doses ranging from 0-300 rads and the animals were then observed until death. Because there were seven active exposure groups plus controls, the study was well designed to demonstrate hormesis. As shown in Figure 2, a hormetic dose-response curve was seen for lung adenoma, but dose-response curves for pituitary and ovarian tumors provide less obvious examples of hormesis. As in the case of cadmium carcinogenicity discussed above, effects in different organs differed markedly.

PAHS

Polycyclic aromatic hydrocarbons (PAH) are among the classic DNA-reactive carcinogens. In studies performed during the 1960s, newborn albino mice were administered a single sc dose of either of two carcinogenic PAHs, 3-methylcholanthrene or dibenz[a,h]anthracene, and observed for up to 79 weeks (O'Gara *et al.*, 1965). Because there were
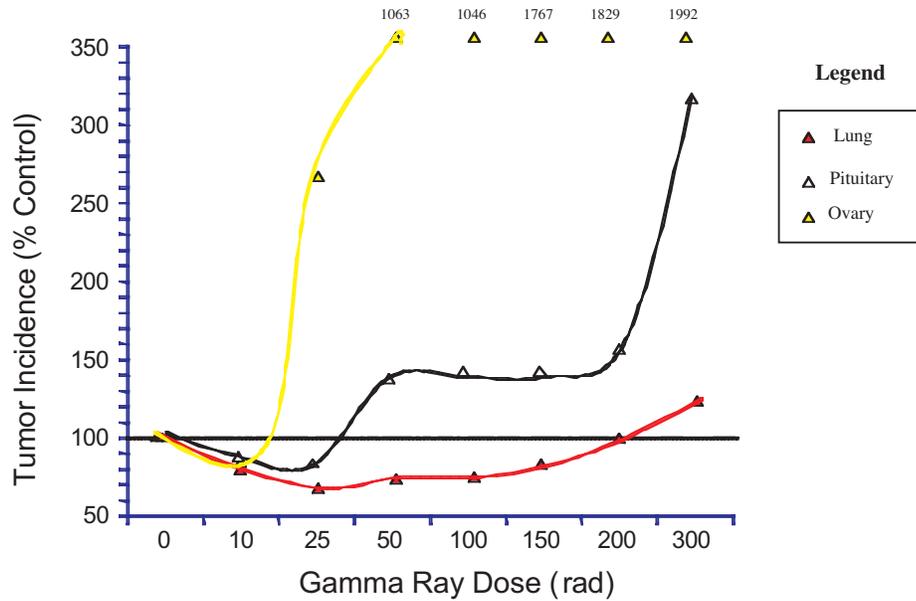
FIGURE 2 Incidence data for tumors of lung, pituitary and ovaries in female RFMf/Un mice exposed to (-irradiation and followed for 104 weeks {22334}. The ovary responses for the five highest doses were above upper bound of the chart.

eight logarithmically-spaced doses plus controls, the study was suitable for demonstrating hormesis. Figure 3 shows dose-response curves for lung tumors in female mice observed 56-79 weeks after dosing. The response curve for 3-methylcholanthrene demonstrate hormesis, but that for dibenz[a,h]anthracene does not. Because the modes of action of these two agents are almost certainly similar, if not identical, and because both presumably evoke similar, if not identical, detoxication and repair mechanisms, such qualitative differences in response are difficult to explain.

2,3,7,8-TCDD

Few toxicants have enjoyed the notoriety of dioxin (2,3,7,8-TCDD) and few have led to greater disputes regarding the nature and extent of their carcinogenic risks to humans. It is generally agreed that dioxin is not DNA-reactive, although it is likely to alter gene transcription. The appropriateness of the linear low-dose extrapolation for the dioxin risk assessment is at the core of ongoing disagreements and lack of definitive regulatory guidance for this pollutant. It has also been proposed that the dose response of dioxin-induced liver cancer is hormetic (Cook, 1994; Calabrese *et al.*, 1999).

It is notable that the same study and same data that EPA has historically relied upon for its dioxin cancer risk assessment are also the study and data cited as evidence of its hormetic dose-response properties. That study (Kociba et al, 1978) exposed male and female Sprague-Dawley rats
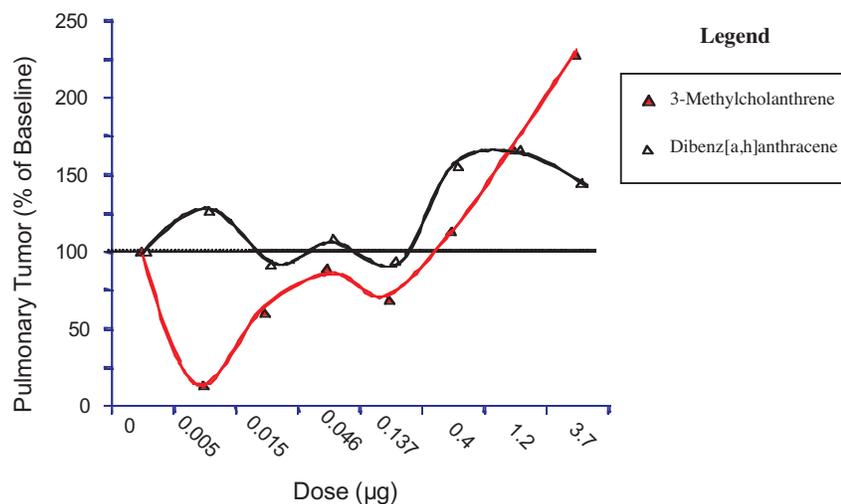
FIGURE 3 Incidence data for lung adenoma in albino mice administered 3-methylcholanthrene or dibenz[a,h]anthracene by single sc injection and followed for 56-79 weeks {22594}.

to nearly pure dioxin mixed in their chow for 2 years. There were three dioxin doses, ranging from 0.001-0.1 (g/kg/day, plus a control group.

Figure 4 shows dose-response curves for liver and pancreas tumors in female rats and Figure 5 shows the corresponding dose-response curves in male rats. (Liver tumors were considered because they are a primary concern of dioxin risk assessments, while pancreas tumors were chosen for illustration; other tumors could have served as well). In females, there was an apparent dose-related increase of liver tumors, but not pancreas tumors. The shape of the dose-response curve for liver tumors in females was compatible with hormesis. In males, the incidences of both liver and pancreas tumors decreased as dioxin doses increased.

Because of the limited number of administered doses, it is difficult to be certain that the dose response curve for female liver tumors is an example of hormesis. If so, then it is challenging to understand why that hormetic response is sex and organ specific. In other words, these data suggest that if a hormetic response is seen in this study, then it must reflect certain peculiarities of the affected organs, rather than being an intrinsic property of the chemical.

DISCUSSION

It seems apparent that some carcinogens demonstrate hormetic dose-response relationships, at least in some studies and under some conditions. It is not known, however, whether such responses are common or uncommon. In addition, the available data do not provide sufficient insight to know whether such hormetic responses are the properties of specific toxic agents, or specific species, or specific organs. From the lim-
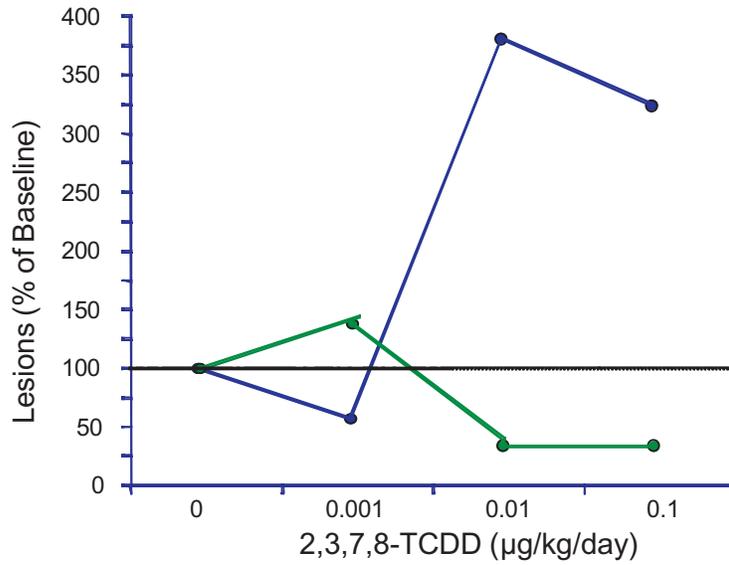
FIGURE 4 Incidence data for liver and pancreas tumors in female Sprague-Dawley rats administered 2,3,7,8-TCDD in their chow for 2 years {22335}{22585}.

ited examples reviewed above, one might infer that the presence of hormesis reflects some complex interaction of all of these considerations. For example, 2,3,7,8-TCDD demonstrated hormesis for female liver tumors, but not male liver tumors and not for other tumors in males or females. Ionizing radiation demonstrated hormesis in some organs, but
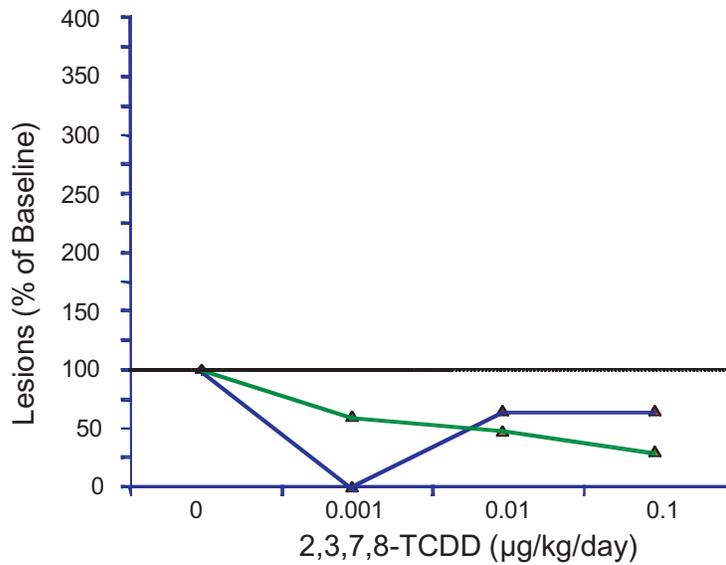

FIGURE 5 Incidence data for liver and pancreas tumors in male Sprague-Dawley rats administered 2,3,7,8-TCDD in their chow for 2 years {22335}{22585}.

not in others, while one of two carcinogenic PAHs demonstrated hormesis but the other did not.

To determine the general relevance of hormesis for carcinogens, it would be necessary to modify the current standard design of cancer bio-assays or initiate additional bio-assays designed to specifically to evaluate the low-dose effects of known and suspect carcinogens. It is not clear that the expense of such testing can be justified readily, at least from the perspective of the public health community. Given the often awesome task of supporting public health initiatives in light of limited public- and private-sector funding, one might expect little enthusiasm for initiating or expanding expensive research projects in order to focus on the hypothetically beneficial effects of low-dose carcinogen exposure.

On the other hand, demonstration of hormetic (or non-linear) dose response relationships may provide important toxicologic insights about underlying mechanisms of carcinogenicity and an organism's mechanisms for adaptation. If hormesis is not a statistical quirk, then it must reflect underlying toxicologic processes and, therefore, can shed light on those factors that interact with and modify a toxicant's mode of action. In other words, the finding of hormesis provides potentially important insights into the actions of toxicants in specific species and organs, insights that may not be gained from exclusively high-dose studies. In addition, the mechanisms that underlie hormesis, especially those due to intrinsic adaptive response, may provide important guidance in the search for better preventive and prophylactic interventions.

The policy implications of hormesis are also complex, especially in light of the current Guidelines. The finding of a hormetic dose-response currently provides no basis for inferring or concluding that a carcinogen's mode of action can be expected to be non-linear at low doses. Likewise, the presence of hormesis is currently not sufficient to argue that body burdens are not so high that incremental doses are expected to affect the linear portion of the dose-response curve. Thus, hormesis provides no obvious alternative to the default linearized low-dose extrapolation model. The issue is whether the finding of hormesis can inform our understanding of mode of action. To that end, the greatest informational value of hormesis may be that it provokes mechanistic studies intended to explain why hormesis occurs. Finally, from a policy perspective (if not a toxicological one), it is important to consider whether the finding of hormesis provides more operationally useful information than the finding of a threshold.

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